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PPARγ regulates the expression of cholesterol metabolism genes in alveolar macrophages

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ABSTRACT

Peroxisome proliferator-activated receptor-gamma (PPARγ) is a nuclear transcription factor involved in lipid metabolism that is constitutively expressed in the alveolar macrophages of healthy individuals. PPARγ has recently been implicated in the catabolism of surfactant by alveolar macrophages, specifically the cholesterol component of surfactant while the mechanism remains unclear. Studies from other tissue macrophages have shown that PPARγ regulates cholesterol influx, efflux, and metabolism. PPARγ promotes cholesterol efflux through the liver X receptor-alpha (LXRα) and ATP-binding cassette G1 (ABCG1). We have recently shown that macrophage-specific PPARγ knockout (PPARγ KO) mice accumulate cholesterol-laden alveolar macrophages that exhibit decreased expression of LXRα and ABCG1 and reduced cholesterol efflux. We hypothesized that in addition to the dysregulation of these cholesterol efflux genes, the expression of genes involved in cholesterol synthesis and influx was also dysregulated and that replacement of PPARγ would restore regulation of these genes. To investigate this hypothesis, we have utilized a Lentivirus expression system (Lenti-PPARγ) to restore PPARγ expression in the alveolar macrophages of PPARγ KO mice. Our results show that the alveolar macrophages of PPARγ KO mice have decreased expression of key cholesterol synthesis genes and increased expression of cholesterol receptors CD36 and scavenger receptor A-I (SRA-I). The replacement of PPARγ (1) induced transcription of LXRα and ABCG1; (2) corrected suppressed expression of cholesterol synthesis genes; and (3) enhanced the expression of scavenger receptors CD36. These results suggest that PPARγ regulates cholesterol metabolism in alveolar macrophages.

Introduction

Pulmonary alveolar proteinosis (PAP) is an autoimmune lung disease characterized by the accumulation of surfactant [1]. Pulmonary surfactant is comprised of 90% lipid, 10% protein, and less than 1% carbohydrate. The lipid component consists of phospholipids and neutral lipids, cholesterol being the major neutral lipid [2]. Alveolar macrophages catabolize and recycle cholesterol from surfactant. However, the alveolar macrophages of PAP patients do not catabolize surfactant sufficiently and become engorged with lipid [3–6]. Cholesterol levels are increased in the lungs of PAP patients [7,8]. Combined with the presence of foam cells in the lungs of patients, these results provided evidence that homeostasis of cholesterol may be disrupted in PAP. Interestingly, the contribution of impaired cholesterol catabolism to the overall disruption of surfactant catabolism in the pathogenesis of PAP has not been specifically addressed.

PPARγ has been implicated as a critical mediator in cholesterol metabolism in various tissue macrophages and PPARγ is constitutively expressed in the healthy lung. We were the first to determine that the alveolar macrophages of PAP patients were deficient in the nuclear transcription factor peroxisome proliferator-activated receptor-gamma (PPARγ) [9,10]. The presence of cholesterol in surfactant suggests that PPARγ may hold particular importance in promotion of cholesterol catabolism in alveolar macrophages and deficient cholesterol catabolism could impair the catabolism of surfactant overall.

Abbreviations: PPARγ, peroxisome proliferator-activated receptor-gamma; ABC, ATP-binding cassette; PAP, pulmonary alveolar proteinosis; GM-CSF, granulocyte–macrophage colony-stimulating factor; BAL, bronchoalveolar lavage; LXR, liver X receptor; ApoE, apolipoprotein E; CYP27A1, sterol 27-hydroxylase; LDL-R, low-density lipoprotein receptor; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; SREBP, sterol regulatory element-binding protein; SRA, scavenger receptor Class A.

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Studies on other tissue macrophages have shown that PPARγ regulates many genes involved in cholesterol transport (influx and efflux) and metabolism. PPARγ regulates the sterol-sensing nuclear transcription factor liver X receptor-alpha (LXRα) and cholesterol transporter ATP-binding cassette G1 (ABCG1) [10–12]. The PPARγ-LXR cascade is critical to maintaining cholesterol efflux in macrophages [13]. CYP27A1 is an important enzyme that converts cholesterol into LXR ligands thereby promoting cholesterol transport from extrahepatic macrophages [14]. Additionally, macrophages have been shown to readily secrete the more polar hydroxycholesterol produced by CYP27A1 in response to cholesterol-loading [15,16].

While deficiencies in cholesterol efflux could lead to cholesterol accumulation [17], the uptake of cholesterol via scavenger receptors has also been strongly associated with the accumulation of cholesterol [18,19]. Scavenger receptors are up-regulated in the presence of substrate yielding macrophages with the unique capacity for cholesterol uptake regardless of intracellular cholesterol levels. The scavenger receptors CD36 and scavenger receptor A-I (SRA-I) internalize cholesterol bound to oxidized (ox)-LDL (reviewed by Glass and Witztum [20]). Macrophages from mice lacking CD36 and SRA-I exhibit a 90% reduction in ox-LDL uptake [18]. Although scavenger receptor-mediated cholesterol uptake is complex and is not fully understood, it is known to be regulated in part by PPARγ: CD36 is directly up-regulated by PPARγ [21] while SRA-I is negatively regulated by PPARγ [22].

PPARγ regulates both the cholesterol influx (CD36 and SRA-I) and efflux genes (LXRα and ABCG1) and therefore influences the level of intracellular cholesterol present in macrophages. In turn, cholesterol levels regulate the de novo synthesis of cholesterol. In response to limited cholesterol, the nuclear transcription factor sterol response element-binding protein 2 (SREBP2) promotes cholesterol synthesis and uptake through the up-regulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) and low-density lipoprotein receptor (LDL-R) (reviewed by Goldstein et al. [23]). Alternatively, intracellular cholesterol and oxysterols negatively regulate cholesterol synthesis in part by reduced transcriptional activity of SREBP2.

To investigate PPARγ and cholesterol catabolism in alveolar macrophages, we have utilized macrophage-specific PPARγ knockout (PPARγ KO) mice which develop a PAP-like lung pathology with the accumulation of cholesterol and foamy, cholesterol-laden alveolar macrophages [24]. The alveolar macrophages of PPARγ KO mice exhibit decreased expression of LXRα and ABCG1 and reduced cholesterol efflux.

These results suggest PPARγ is a key mediator of cholesterol catabolism in alveolar macrophages. Thus, we hypothesized that in addition to dysregulated cholesterol efflux genes, the expression of cholesterol synthesis and influx genes are also dysregulated in the alveolar macrophages of PPARγ KO mice. To test this hypothesis we investigated the expression of cholesterol metabolism genes and the effects of *in vivo* replacement of PPARγ using a Lentivirus expression system (Lenti-PPARγ).

Materials and methods

Mice. Animal studies were conducted in conformity with Public Health Service policy on the humane care and use of laboratory animals and were approved by the Institutional Animal Care Committee. C57Bl/6 wild type mice were obtained from Jackson Laboratory (Bar Harbor, ME). Macrophage-specific PPARγ KO mice have been previously described [25]. BAL cells were obtained as described earlier from 8 to 12 week old PPARγ KO mice and age- and gender-matched wild type C57Bl/6 controls [25]. For experiments, four or more individual PPARγ KO mice were used except where indicated. For wild type mice, three or more sets of pooled cells from 3 to 5 mice were used in all experiments. Briefly, the thoracic cavity was opened and the lungs were exposed. After cannulating the trachea, a tube was inserted and BAL was carried out with warmed (37 °C) PBS in 1 mL aliquots. Cell viability was measured by trypan blue exclusion. BAL cell differentials from all animals used in the experiments were stained with a Wright-Giemsa stain and revealed >90% macrophages.

Lentivirus plasmid and instillation. A self-inactivating Lentivirus expression vector containing cDNA corresponding to the human PPARγ (Lenti-PPARγ) was cloned into the multiple cloning sites downstream of a CMV promoter using standard techniques as described [25,26]. A Lentivirus expressing the enhanced Green Fluorescent Protein (Lenti-eGFP) was obtained using a similar protocol and was utilized as a control. For experiments utilizing Lenti-eGFP and Lenti-PPARγ, mice were intra-tracheally instilled 30 days prior to BAL, as described previously [25].

RNA purification and analysis. Total RNA was extracted from the cells by the RNeasy protocol (Qiagen, Valencia, CA). Expression of mRNA was determined by RT-PCR analysis using the ABI Prism 7300 Detection System (TaqMan; Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. RNA specimens were analyzed in duplicate using primer sets for mouse ABCA1 (Mm00442646), ABCG1 (Mm00437390), CD36 (Mm00432403), HMGCR (Mm01282494), LDL-R (Mm00440169), LXRα (Mm00443454), LXRβ (Mm00437262), SRA-I (Mm00446214), and SREBP2 (Mm01306300) (Applied Biosystems). Threshold cycle values for genes of interest were normalized to a housekeeping gene (GAPDH, 4352339E) (Applied Biosystems) and used to calculate the relative quantity of mRNA expression in PPARγ KO samples compared with wild type murine controls. For the Lentivirus experiments, Lenti-eGFP samples were used as the controls for Lenti-PPARγ samples. Data are expressed as fold change in mRNA expression relative to control values [27].

Statistical analysis. Data were analyzed by Student's *t*-test using Prism software (GraphPad, Inc., San Diego, CA). Significance was defined as $p \leqslant 0.05$. Results are expressed as mean ± SEM.

Results and discussion

Cholesterol metabolism genes are dysregulated in PPARγ KO alveolar macrophages

We have previously shown that the alveolar macrophages of PPARγ KO mice exhibit reduced cholesterol efflux and are loaded with cholesterol [24]. The lungs of PPARγ KO mice also have elevated levels of cholesterol. Therefore, we investigated the expression of cholesterol genes that are regulated by intracellular and extracellular cholesterol in the alveolar macrophages of PPARγ KO mice.

RT-PCR analysis of SREBP2 and downstream targets HMGCR and LDL-R in the alveolar macrophages of PPARγ KO mice demonstrated that the expression of SREBP2, HMGCR, and LDL-R was significantly decreased (1.8-fold, 1.5-fold, and 1.9-fold, respectively) compared to wild type (Fig. 1A). Alternatively, the expression of scavenger receptors CD36 and SRA-I which are positively regulated by extracellular cholesterol content, specifically cholesterol bound to ox-LDL [20], was up-regulated 1.5-fold and 10.1-fold, respectively (Fig. 1B).

While the regulation of cholesterol synthesis genes is complex, cholesterol has been shown to negatively regulate cholesterol synthesis at the transcriptional level [28]. Wang et al. demonstrated that the accumulation of free cholesterol in ABCG1-deficient macrophages inhibited the expression of HMGCR and LDL-R [29]. THP1-derived macrophages loaded with ox-LDL displayed reduced HMGCR mRNA [30]. Moreover, incubation of murine macrophages with modified-LDL decreased HMGCR enzymatic activity [31].